

Embryonic development as a quasi-historical process

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ABSTRACT This essay explores the nature of embryonic development in contrast to other kinds of processes. Anhistorical processes are highly reproducible and are therefore subject to standard scientific analysis. Such analyses yield results that may well apply universally. Euhistorical processes are non-reproducible. Therefore they are not subject to standard scientific analysis, but are investigated primarily by retrospective speculation. Information gained from such analyses is of relatively limited applicability. Embryonic development exhibits traits associated with both anhistorical and euhistorical processes and is therefore defined as a quasihistorical process. The quasihistorical nature of developmental processes places constraints on the nature of the solution we can hope to obtain for the problem of development, but also provides a means of exploring the euhistorical process of evolution.

KEY WORDS: *development, history, evolution*

Introduction

As I reached the stage in my career at which I needed to begin giving seminars, I found it difficult to rationalize to myself, and thus to a prospective audience, just why I was studying cell fate determination in leech embryos, instead of some other process in some other animal. At that time, the best I could do was to skirt the issue by asserting that "The central question in developmental biology is, 'What is the central question in developmental biology?'"

The line drew a nervous laughter that I may not have fully appreciated at the time. I eventually got a job, but the question of how my work relates to that of other developmental biologists, and how developmental biology as a whole relates to other intellectual enterprises, has remained a source of frustration and entertainment for the past 15 years. Over this time, I have assembled a response to this question that I offer to students in my developmental biology classes, to prospective postdoctoral fellows and to visiting colleagues. Without meaning to suggest that there is anything original in either my analysis or my conclusions, I find that they serve the purpose of keeping my lecture classes and research group at manageable sizes and insuring that my schedule is not overly burdened with seminar invitations. Such encouragement notwithstanding, I offer this version of the rationalization in honor of the discussions between Antonio García-Bellido, Gunther Stent and other true scholars that I have had the good fortune to overhear.

The key to my argument is that there is not one but two "central questions" in developmental biology. More precisely, I see two *classes* of questions, one mechanical, for which the general outline of the solution is already in place, and the other historical, to which scientists are just returning after a long absence.

Development as a mechanical process

The first class of questions is exemplified by one attributed to Georg von Lichtenburg (1742-1799) by J. G. Nicholls (personal communication), "How is it that the cat has two holes cut in its skin at just the same spot as its eyes are?" Such questions are concerned with particular mechanisms of development in some particular species of animal. A general form of this question would be "How is it that any one species develops from egg and sperm to adult?"

For anyone observing a developing embryo twenty years ago, the intricate machinery of embryonic cell divisions, movements, growth and differentiation was so overwhelmingly complex that this question would seem unanswerable or even unapproachable. But the embryonic development of *Drosophila melanogaster* is now well understood at a level of detail undreamed of even a decade ago, and the progress continues unabated. This tremendous achievement is thanks to the convergence of powerful genetic and molecular techniques plus a massive long term research effort representing hundreds of thousands of man-years devoted to the biology of this single species. One key step was the identification, through systematic mutagenesis, of about 120 genes that are required to control embryonic pattern formation in *Drosophila* (Nüsslein-Volhard and Wieschaus, 1980). Many, if not most of these genes and their products, have already been cloned, sequenced and characterized in terms of expression pattern, probable biochemical function and genetic interactions. Moreover, thanks to recent advances and the size of the community of *Drosophila* biologists, it is now feasible to extend such studies to all 1200 of the embryonic lethal genes that have been identified, if so desired.

Abbreviations used in this papers: NTP, nucleotide triphosphate.

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The incredible wealth of detailed information regarding the development of *Drosophila* is satisfying in and of itself, and here we honor Antonio García-Bellido's many contributions to this body of work. While there are new genes that remain to be discovered and characterized, it seems unlikely that there will be any radical changes to our current understanding of how embryos work: cells are born with a particular mixture of protein and RNA species that influence their subsequent intrinsic transcriptional and translation activity. In addition to this intrinsically determined course, cells are subject to extracellular influences that activate intracellular signal transduction pathways, initiating new transcriptional, translational and post-translational processes. García-Bellido's distinction between selector and realizator genes, and his conceptualization of genetic syntagmata (García-Bellido, 1985) have provided an essential intellectual framework for managing this mass of information, just as the discovery of the developmental compartments (García-Bellido *et al.*, 1973) provided an essential part of the experimental framework for generating it.

One of the least well understood areas in developmental biology (of *Drosophila* or any other organism) is that of how the biochemical state of the cell determines macroscopic cell behaviors such as motility, shape change and progression through the cell cycle, and how complex behaviors at the tissue level emerge from populations of cells. It also remains to be seen to what extent the interactions between numerous complex signaling and transcriptional pathways that affect the course of development can be reduced to a comprehensible genetic and biochemical circuit comparable to the circuit diagram for an electronic device. The alternative is that, while the individual circuit elements and their connections can be identified, the overall circuit is so complex that its function cannot be deduced by inspection, but only by computer modeling. This situation is familiar to neurobiologists studying the neural circuits of "simple" invertebrate behaviors (Brodfuehrer *et al.*, 1995).

These two grey areas notwithstanding, it seems fair to say that, to the extent that the central question of developmental biology is "How does an organism, *any* organism, develop?", the answer, while not completely elaborated, is near at hand; the prizes have been awarded (Lewis *et al.*, 1996) and the book has been written, at least in its first edition (Lawrence, 1992).

But does this great scientific triumph "solve" development in the sense that knowing the structure of DNA and the central dogma of molecular biology solved the problem of heredity? Indeed, given the diversity of embryo types and adult body plans, can we even say that a general solution may exist that is not so vague as to be useless? If so, what is it and if not, why not?

Clearly, we can only approach these issues by identifying which parts of the *Drosophila* solution apply to other organisms. It turns out that these issues lead to the second class of "central questions" in developmental biology, which are partly historical in nature, best exemplified by the brain teaser "Which came first, the chicken or the egg?" In considering these questions, we come to understand that limits to our ability to "solve" development arise ineluctably from its "quasi-historical" nature. To explain what this means, let us first define two other types of processes, namely "anhistorical" and "euhistorical" processes.

Anhistorical and euhistorical processes

An anhistorical process is one that is highly reproducible, meaning that we can predict the outcome with great accuracy

knowing the starting conditions. In addition, it is minimally contingent, in the sense that it is largely independent of previous events in the system. Consider two colliding hydrogen atoms. The distribution of possible outcomes from the collision can be predicted with confidence knowing nothing more than that the colliding particles are hydrogen atoms and their relative velocities. The outcome distribution is the same for atoms that have been traveling alone through space for 100 million years prior to their collision as for those coming fresh from a molecule of cholesterol.

Because it is reproducible and minimally contingent, the anhistorical process is subject to rigorous scientific analysis. Chemical physicists can study the mechanisms of the reaction by changing the conditions and observing the changes in the outcome of the process. Moreover, in the view of most scientists, the nature and outcome of the reaction are no different here in the solar system than on the other side of the universe, and no different now than 20 billion years ago. As far as we know, the knowledge obtained from studying anhistorical processes is literally timeless and of universal applicability.

A euhistorical process, in contrast, is one that shares certain key features with what we normally think of as history. [Thus, I am using the term euhistory in the same sense that the term "analog watch" is used to designate what was originally called simply a "watch".] In contrast to the process of reacting hydrogen atoms, euhistorical processes are fundamentally non-reproducible and highly contingent. That is, they are critically dependent on preceding events, some of which are themselves determined by random variation and could never be recreated. Figures in human history are not interchangeable in the sense of the colliding hydrogen atoms and therefore we do *not* repeat history, whether we study it or not. There was only one Napoleon Bonaparte. We can ask how world history might have unfolded if he had been a foot taller, but we can do no better than to make informed but inconclusive speculations as to the answer, for two reasons. First, it is impossible to test our speculations by re-establishing all the critical starting conditions at some point and then changing a single variable (e.g., Napoleon's height). And second, even if we tried, the outcomes of historical processes (e.g., Napoleon's invasion of Russia) are contingent upon factors (e.g., the winter weather conditions) that are themselves determined by chaotic processes and probabilistic events that would not repeat exactly even if the starting conditions were identical.

One consequence of the non-reproducible and highly contingent nature of euhistorical processes is that the mechanisms involved are not subject to scientific analysis, but rather only to retrospective speculations. Moreover, the results obtained from analyzing history are of limited and uncertain applicability. Applying the lessons learned from the trench warfare of WWI in constructing the Maginot Line, France was left vulnerable to the new strategies of warfare that emerged in WWII.

Defining euhistorical processes as those for which important aspects of the outcomes are non-reproducible and highly contingent, for which analysis by controlled experimentation is largely replaced by speculation after the fact, and for which any results obtained are applicable locally rather than universally, we see that the category of euhistorical processes includes not only human history but also processes such as stellar, planetary and biological evolution. With respect to biological evolution, for example, the stories of how various species arise, change, survive and go extinct hang on chance events ranging from mutations at the molecular

scale to asteroid impacts at the global scale and are thus non-reproducible in major aspects (e.g., Gould, 1989). In addition, the features of each new species that arises are derived by modification of preexisting species and thus are highly contingent on the features of the ancestral species (Jacob, 1982).

For these reasons, the validity of our understanding of the actual events of evolution results cannot be proven by controlled scientific experiments of the sort used to analyze the reaction of the colliding hydrogen atoms. Moreover, whatever information we are able to glean is of restricted applicability both temporally and spatially. As far as we know, life of any sort is a rare and recent phenomenon in the universe. Of the myriad of possible chemical structures and reactions possible, only a tiny set actually occur in organisms living on earth. Even if carbon-based life of approximately the same biochemical basis has evolved elsewhere, it seems highly unlikely that it would be the same as life on earth because life on earth represents just one point in a hugely dimensioned space of contingent outcomes.

Development as a quasi-historical process

If we now consider the process of embryonic development, we find that it is a hybrid, sharing properties of both anhistorical and euhistorical processes. On the one hand, development is reproducible, in that each zygote of a species normally gives rise to the larva or juvenile of that species. Because of this reproducibility, the mechanisms by which the process proceeds can be investigated by systematic experimentation, as has been demonstrated so elegantly in the case of *Drosophila*. For this purpose, we use the same scientific approach, albeit with different experimental techniques, as are used to understand the reactions of the colliding hydrogen atoms. In this sense then, the process of embryonic development resembles anhistorical processes. But the developmental mechanisms that we seek to understand are highly contingent and of greatly restricted applicability, characteristic of euhistoric processes. For instance, processes operating at one time during development are set in motion by those operating earlier and set the stage for those that follow. Moreover, the developmental processes in each species are the product of evolutionary "tinkering" by which that species has arisen from its ancestors (Jacob, 1982). Thus, developmental processes cannot be understood as being the optimal way of achieving some structure or function in an organism *de novo*. Rather, they represent the non-lethal results of random changes in processes present in the ancestral species. Thus, the details of any developmental process we might study are severely constrained by and embedded in the euhistorical process of evolution.

Obviously then, many of the important details of developmental processes are of limited applicability, not only because they apply (so far as we know) only to earth-based life, but also because they apply only to a particular species or narrow group of species and will become mute once the animals in question go extinct. This combination of properties associated both with euhistorical processes (highly contingent and limited applicability) and anhistorical processes (reproducible and subject to rigorous mechanistic analysis by scientific experimentation), marks embryonic development as a quasihistorical process.

Of course, every biochemical process is quasihistorical. Amino acids and sugars can be synthesized in the laboratory from simple starting materials by numerous routes. At most only a few of these occur in living things and of course, these molecules must have

arisen first by non-biological pathways, wherever they arose. Of all the conceivable synthetic routes, the ones followed in living cells, using specific biomolecules as the source of chemical energy and redox potential, along with incredibly specific, hugely complex polypeptides as catalysts, are obviously contingent upon the historical process of evolution. Each biosynthetic pathway represents just one of many possible routes that could have evolved and could have served just as well, given different combinations of chance events during biogenesis.

But the historical component of development processes is more pronounced than for basic biochemical processes. The level of contingency is higher, because the species-specific aspects of developmental processes are often of greater significance than the general principles. For example, DNA replication is very much the same between different organisms. The raw materials (NTPs) are identical and the DNA templates are interchangeable for the replication machineries of different species. True, there are amino acid substitutions between the polymerase molecules of different species, and studying these may be of use for understanding the structure and function of the enzymes, but polymerization invariably occurs by addition at the 3' end of the chain. Thus, there seems little to be gained from studying DNA replication in large numbers of species.

In contrast, it is impossible to distinguish general aspects of developmental processes from species-specific ones by studying one or even just a few organisms. Because there is so much variability at the cellular level from one kind of animal to another, it's hard to be sure which parts of the process are truly general and which are unique to the embryos of a particular taxonomic group or morphological type. Moreover, the generalities we can draw are unsatisfactory as explanations of development without species-specific details. For example, we can say that gastrulation entails the internalization of mesodermal and endodermal precursors, followed by the elaboration of the long axis of the embryo. But in different species, the internalization occurs by ingression, invagination, delamination, involution, epiboly or some combination of processes, and the elongation can result from convergent extension (which may itself involve separate processes of radial and mediolateral intercalation), oriented cell division, or other processes. Moreover, there is no fixed relationship between internalization and elaborating the long axis. In *Xenopus laevis*, internalization and elaboration of the long axis are so inextricably linked that it is hardly possible to think of them as distinct processes, whereas in *Drosophila* the anteroposterior axis is essentially complete upon cellularization, prior to any internalization of mesodermal and endodermal precursors. Thus, because of the quasihistorical nature of development, understanding gastrulation in *Xenopus* is not sufficient to explain gastrulation in other animals, and if we seek a general solution for development, we seek in vain.

Development and evolution

In the pre-Darwinian era, it was commonly assumed that all species of life were created more or less simultaneously (within a one week period according to the Judeo-Christian mythology) and that species are immutable (observations on breeding and hybridization of domestic plants and animals notwithstanding). Within this frame of reference, while comparative studies in morphology, behavior or embryology might be undertaken, they had no more

intrinsic intellectual merit than a "compare and contrast" question on the final exams for an undergraduate literature course. This situation changed with the realization in the 19th century that all modern species have arisen by divergent modification of ancestral ones. At that point, comparative studies took on a central importance as a way of sorting out the phylogenetic relationships between modern species and also between modern species and ancestral ones.

This interest in comparative biology was focused onto comparative development by Haeckel (1866), who proposed that in development, each organism undergoes a shortened and accelerated recapitulation of its evolutionary history. Given the vague and oversimplified nature of the starting premise, and the lack of techniques with which to investigate developmental mechanisms, it is not surprising that comparative embryology did not lead to any great insights. In the twentieth century, the field was eclipsed by reductionist and fruitful biological disciplines, such as physiology, biochemistry, genetics and eventually molecular biology, subfields in which, as we have discussed above, the historical component is of less significance.

With success in these fields, biologists turned their attention to development, with the anticipation by some that it would yield like a ripe fruit to the central dogma of "DNA makes RNA makes protein". The most ambitious proposal was that the DNA would encode a complete program, containing all the information required to understand the development of the organism. This notion was criticized by Stent (1981), who pointed out the importance of the (historically derived) cellular context within which we interpret DNA sequences.

As described above, while molecular genetic analysis of *Drosophila* development has been immensely successful, it has not provided a universal, or even a global "developmental code" that obviates analyses of other types of embryos. On the contrary, the elucidation of *Drosophila* development has reinvigorated comparative studies, as a means for distinguishing those aspects of the developmental mechanisms that are likely to be broadly conserved, as distinct from those that are species- or taxon-specific, and also for understanding how one species arose from another.

The realization that many of the developmental regulatory genes identified in *Drosophila* are evolutionarily conserved, together with the application of molecular techniques sometimes referred to as "reverse genetics", provides us with powerful new tools for studying the development of other species that are not amenable for genetic analysis. Genes homologous to developmental regulatory genes of *Drosophila* exist in other species, and they encode proteins whose biochemical properties are similar to those of their *Drosophila* homologs. But given the quasihistorical nature of developmental processes, the question remains as to what embryological functions these molecules play in different types of embryos and this information can only be obtained by comparing developmental processes of diverse taxa (including leeches!). By this means, we can hope to discern which aspects of developmental mechanisms in *Drosophila* are generally applicable.

Another function of comparative studies of development is to reveal those aspects of developmental mechanisms that are spe-

cific to the various taxa, thus providing insights into how developmental mechanisms have changed over time, and how those changes in developmental mechanisms have given rise to the diverse body plans represented in modern species. For this purpose, we seek to interpret comparative studies in light of known phylogenetic relationships. Here we run into another problem. Traditional phylogenetic trees have been generated by comparing representatives of various taxa on the basis of whether or not they share one or more "characters". Often the traits compared are overtly embryological in nature (e.g., cleavage patterns). Even behavioral or morphological traits have their basis in developmental processes.

But this means that we have been trying to compare developmental processes in light of phylogenetic trees that already have developmental comparisons built in; there is an unpleasant circularity to the logic of this approach. Here, too, molecular biology has made an important contribution, by allowing us to construct trees that are based on sequence comparisons. Such trees are relatively independent of any developmental constraints, especially using the genes such as RNA polymerase (McHugh, 1997), whose highly conserved biochemical functions insulate them from the influence of developmental variations. By comparing developmental mechanisms in light of independently established phylogenetic trees, developmental biologists become natural historians in the truest sense of the term, trying to reconstruct the course of evolution by speculating about the nature and the sequence of changes in developmental mechanisms over time.

Thus, because development is a quasihistorical process, the knowledge gained from studies of development are inextricably linked to the evolutionary history of the species under investigation. This limits our ability to "solve" the problem of development, but it also provides a window (heavily frosted though it is) into the euhistorical process of evolution.

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